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RESEARCH PAPER

Cytochemical localization of ATPase and sub-cellular variation in mesophyll cell of *Cyclocarya paliurus* seedlings under iso-osmotic stress and calcium regulation

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Abstract: The ultrastructural distribution and active location of ATPase and the ultrastructural variations were investigated in mesophyll cells of *Cyclocarya paliurus* seedlings after iso-osmotic salt/water treatments in combination with calcium regulation. *C. paliurus* seedlings were treated with five groups (control, 85 mM NaCl, 85 mM NaCl + 12 mM $Ca(NO_3)_2$, PEG iso-osmotic to 85 mM NaCl and PEG iso-osmotic to 85 mM NaCl +12 mM $Ca(NO_3)_2$) in a hydroponic system in a phytotron. Results show that under normal growth conditions, the ATPase activity was low and the enzyme was primarily located on the nucleus. After 12 days of iso-osmotic salt/water treatments, ATPase activity on the tonoplast increased. Osmiophilic globules for iso-osmotic water treatment were greater than that for iso-osmotic salt treatments. The ATPase activity increased and was mostly transferred onto the nucleus for calcium regulation treatment under iso-osmotic salt/water stresses, and the osmiophilic globules significantly decreased under iso-osmotic water stress with calcium regulation. The ATPase located on the nucleus indicated that the degree of salt/drought damage that seedlings suffered was slighter, while the amount of the enzyme located on the tonoplast showed that the degree of salt/drought damage there was more serious. After 4 and 20 days of iso-osmotic treatments, the injury suffered by the leaf ultrastructures of *C. paliurus* seedlings for iso-osmotic treatment with calcium regulation was lower than those without calcium regulation, especially for the iso-osmotic water treatments. Preliminary analysis suggests that the injury suffered by *C. paliurus* seedlings was lower for iso-osmotic salt treatments than for iso-osmotic water treatments, while the effect of calcium regulation under iso-osmotic water stress was greater than that of the iso-osmotic salt stress.

Keywords: ATPase; calcium regulation; *Cyclocarya paliurus*; cytochemistry; iso-osmotic stress; mesophyll cell; ultrastructure

Introduction

The enzyme class ATPase fulfills a wide range of important functions, including catalysis of the hydrolyzation of Adenosine triphosphate (ATP), participation in the transport of materials and ions, cellular signal transduction, and many other kinds of metabolic activities (Hasegawa et al. 2000; Chen et al. 2006; Christian et al. 2006). As we known, there are many kinds of proton or cation transporting ATPases in plant cells, and these enzyme ATPases play central roles in maintaining the pH value of the cytoplasm, Ca^{2+} concentrations, water potential and the stabilization of intracellular metabolism (Martinez et al. 2003; Salem et al. 2005). Cytochemical methods are powerful tools for the precise mapping of the distribution of ATPase, and are able to localize its activity at the ultrastructural level *in situ* (Wang and Sze 1985; Morsomme and Boutry 2000; Wang et al. 2001; Shi et al. 2003; Qiu et al. 2004; Yang et al. 2007). So far little is known about the distribution and the locations of activity of this enzyme in woody plants under adverse circumstances or stress (Yang et al. 2007).

Ca^{2+} is of great importance to plant metabolism. Ca^{2+} is not only acted as an ingredient made of cell, but as the second messenger to regulate plants in response to environmental changes. Yao and Fang (2007) found that under osmotic stress, exogenous Ca^{2+} could constrain roots from absorbing redundant Na^{+}, and could effectively relieve drought stress in *Cyclocarya paliurus*. However, few work is conducted to compare the effects of calcium regulation under iso-osmotic salt and water stress.

Cyclocarya paliurus (Batal) Iljinskaja (family Juglandaceae), a native to China, is the sole species in its genus and grows at a range of about 420–2 500 m elevation in the mountainous regions. In China, the bark and leaves of *C. paliurus* are widely

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used for making medicinal tea (Xie and Li 2001; Fang et al. 2006). A new crystalline compound, called cyclocaric acid A (3,23-*β*-epoxy-olean-12- en-28-oicacid), was isolated from the leaves of *C. paliurus* (Fang and Fu 2007). In addition to the new compound, a rich polysaccharide content from leaves of *C. paliurus* was confirmed, which is effective in reducing blood glucose and improving the capacity of glucose tolerance in diabetic mice (Xie and Li 2001; Li et al. 2002). An *in vitro* study showed that *C. paliurus* inhibits alpha-glucosidase, a disaccharide-degrading enzyme in the small intestinal mucosa, leading to a decrease in the absorption of 4 glucose in the blood and a subsequent lowering of the blood glucose level (Kurihara et al. 2003). Xie and Li (2001) reported that the contents of the flavonoids vitamin E (VE) and vitamin C (VC) in the leaves of *C. paliurus* were higher than that in other plants. A huge production of tender leaves from *C. paliurus* is required for the raw material for teas and medicinal use, thus increasing demands for leaf production and for new plantations are anticipated.

Worldwide potential agricultural use is approximately 380 million hectares, but in parts of locations the productivity is severely restricted by salinity (Lambers 2003). There is a total of about 27×10^6 ha of saline soil in China, of which coastal land accounts for 8% (Yao and Fang 2007). These land resources have great potential for developing forestry. The injury suffered by plants exposed to sodium chloride is considered to be the result of both ionic damage and osmotic damage due to a lowered water potential (Zhao et al. 2003). The two factors are interrelated and coexist under saline soil conditions. The dual stresses in common lead to some confusion in the distinct physiological and ecological responses of plants (Zhao et al. 2003). Plants for growth or survival must possess the ability to counter both stresses simultaneously. To our knowledge, no information is available on the salt and drought tolerance for *C. paliurus* under iso-osmotic salt and water treatment. Mechanisms by which plants tolerate salt and drought are easily confused under iso-osmotic treatment and they differ from species to another (Greenway and Munns 1980; Ashraf and Harris 2004). In this study the cytochemical localization of ATPase and sub-cellular variation in mesophyll cells of *C. paliurus* seedlings under iso-osmotic stress and calcium regulation were studied. The objectives of this study were (1) to investigate ATPase distribution and the active location of this enzyme, (2) to examine the ultrastructural variation of ATPase, and (3) to evaluate the salt and drought-tolerance of *C. paliurus* seedlings, and the effects of calcium regulation on plants response to iso-osmotic salt and water stress. The present study contributes to mechamism comparisons of plants in response to salt and water stress and enhances understanding of the relationship between the ATPase and the salt and drought-tolerance capacity of *C. paliurus*.

Materials and methods

Plant materials and growth conditions

Seeds of *Cyclocarya paliurus* were collected in October 2004 from Ganzhou in Jiangxi Province, China. Seed trees were about 15 m tall and 28 cm in diameter at breast height. After air drying and removing extraneous matters, the seeds were stratified in pails according to the size ratio of 1 (seed) to 3 (sand, mixed with gibberellins), under natural conditions in order to break seed dormancy. The stratification started in January 2005 and ended in March 2006. Seeds were sowed in containers with mixed medium (perlite: vermiculite: peat soil=1:2:2) after they were stratified. When the height of the seedlings reached about 7 cm, they were transplanted into black plastic boxes with 1/2 strength Hoagland nutrient solution. The size of the plastic box was 56 cm (length) ×36 cm (width) ×26 cm (height), and the thickness of the plastics was 8 mm. After 10 days of cultivation, uniform seedlings were selected and placed in plastic boxes with the Hoagland nutrient solution containing the different iso-osmotic treatments.

In July 2006, plant materials were divided into five groups for different iso-osmotic stress and calcium regulation treatments: (1) Hoagland's nutrient solution (as CK); (2) 85 mM NaCl (S); (3) 85 mM NaCl + 12 mM $Ca(NO_3)_2$ (SC); (4) PEG iso-osmotic to 85 mM NaCl (W); (5) PEG iso-osmotic to 85 mM NaCl +12 mM $Ca(NO_3)_2$ (WC). Each treatment was applied to 30 seedlings. The solutions were aerated throughout the experiment, the volume was maintained by adding water to compensate for water loss by evaporation and transpiration, and the nutrient solution was renewed every 7 days from when the seedlings were transplanted into the boxes. The seedlings were grown in the phytotron at a photon flux density of 350–400 $\mu mol\cdot m^{-2}\cdot s^{-1}$ in 12 h each day, and the temperature was 26±0.5/20±0.5 with a relative humidity of 70%/80% in a day/night cycle.

Cytochemical localization of ATPase

The ultrastructural enzyme cytochemical technique involved the use of a heavy metal simultaneous capture reaction (Joshi et al. 1988) and was a modification of that reported by Chen et al. (2006). Briefly, after 12 days of salt treatment, leaves from the same position were cut into fractions of about 0.3 mm^3, and rapidly put into 2.5% glutaric dialdehyde and 4% paraformaldehyde, and fixed for 2 h at 4°C, washed four times (half an hour each) with 50 mM cacody- late buffer. The remnant was washed with glutaric dialdehyde with 50 mM tris-maleate buffer, pH 7.2, then incubated for 2 h at 37°C. The incubation medium was made up of 50 mM tris-maleate buffer (pH 7.2), 2 mM ATP (Na^+ salt), 3 mM $Pb(NO_3)_2$, 5 mM $MgSO_4.7H_2O$ and 1% sucrose. The controls were the same as the incubation medium, but without the addition of ATP (Na^+ salt). After a 2 h incubation, the samples were washed two times at 4°C with 50 mM tris-maleate buffer (pH 7.2), and rinsed briefly with 50 mM cacody- late buffer, pH 7.2. They were fixed with 1% osmium tetroxide confected with 0.1 M cacody- late buffer (pH 7.2) for two hours at 4°C, then washed four times with 50 mM cacody- late buffer. Finally, samples were dehydrated in an alcohol gradient, passed through propylene oxide, embedded in Epon812, and then sliced using an LKB-2088 type ultramicrotome. Sections were not stained with uranium acetate and lead citric acid, investigated and photo-

graphed under transmission election microscope of HITACHI H-600 type.

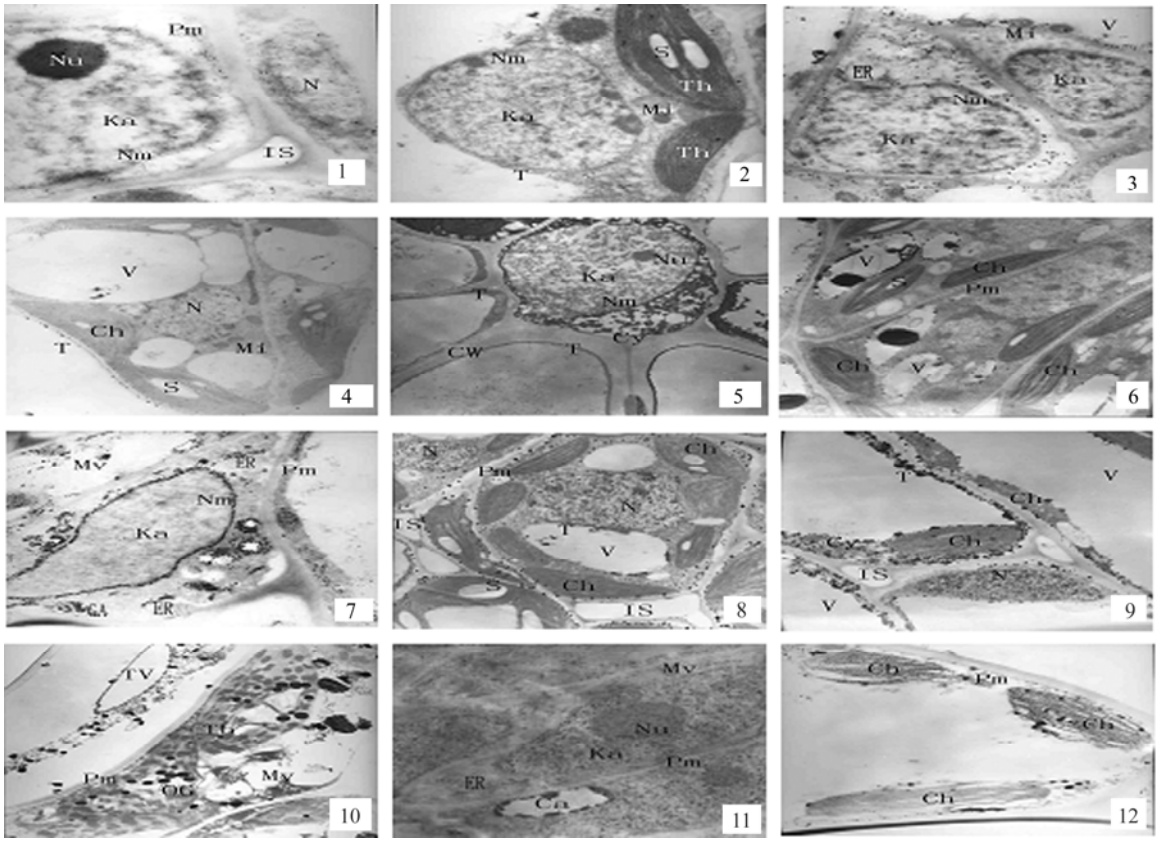
Investigation of ultrastructural variation

The investigation of the ultrastructural variation was conducted as described by Peng et al. (2004) with some modifications. After 20 days of salt treatment, fractions of about 1 mm^2 of leaves collected from the same position were quickly put into 3% glutaric dialdehyde with 0.1 M phosphoric acid buffer, pH 7.0, fixed for 3 h at 4°C, and washed three times (half an hour each) with the same buffer. They were then fixed in 1% osinic acid confected with 0.2 M phosphoric acid buffer (pH 7.0) for 24 h at 4°C, then washed three times with the same buffer solution. After the wash, samples were dehydrated in an alcohol gradient, passed through propylene oxide, embedded in Epon812, then sliced using LKB-2088 type ultramicrotome. Sections were stained with uranium acetate and lead citric acid, observed and photographed under transmission election microscope of HITACHI H-600 type.

Results

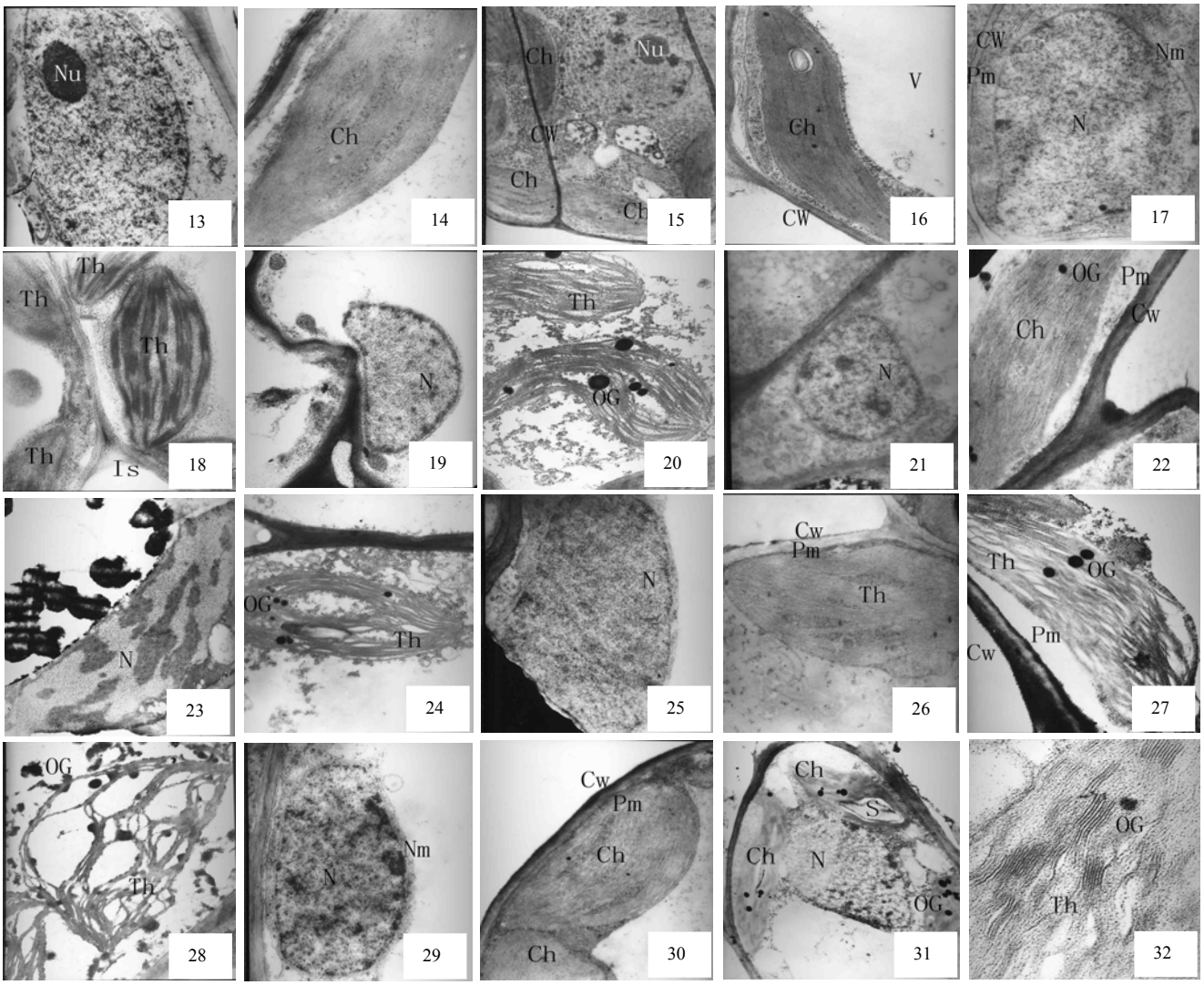
Cytochemical localization of ATPase in mesophyll cell under iso-osmotic treatments and calcium regulation

To clearly investigate the distribution of ATPase, and not confuse it with electric staining or artificial feint, ATPase activity could be verified only by comparison with controls. The results by comparison with controls with or without the addition of ATP (Na^+ salt) showed that no lead phosphate deposits were found in the controls without the addition of ATP (Na+ salt) (Figs. 1 and 2). Lead phosphate deposits were found without diffusing phenomena in the controls with the addition of ATP (Na^+ salt) (Figs. 3 and 4). This indicated that the results seen in the Figures are credible.



Figs. 1-12. Localization of ATPase activity in mesophyll cell of *C. paliurus* seedlings after 12 days of iso-osmotic NaCl and water stress. Fig. 1 No ATPase activity reaction in vein cell for CK treatment without substrate addition, ×25000. Fig. 2 No ATPase activity reaction in palisade cell for CK treatment without substrate addition, ×10000. Fig. 3 ATPase activity reaction on nuclear membrane and endoplasmic reticulum of vein cell for CK treatment, ×12000. Fig. 4 ATPase activity reaction on tonoplast of palisade cell for CK treatment, ×6000. Fig. 5 ATPase activity reaction on nuclear membrane and tonoplast of vein cell for S treatment, ×6000. Fig. 6 A little of ATPase activity reaction on tonoplas of palisade cell for S treatment, ×8000. Fig. 7 ATPase activity reaction on the endoplasmic reticulum, golgi apparatus, multivesicle bodies and nuclear membrane of vein celll for SC treatment, ×15000. Fig. 8 Obvious grain of ATPase activity reaction on karyotin, cytoplasm and tonoplast of palisade cell for SC treatment, ×6000. Fig. 9 Much of ATPase activity reaction on karyotin, cytoplasm and tonoplast in vein cell for W treatment, ×8000. Fig. 10 ATPase activity reaction on topnoplast of palisade cell for W treatment, and appearing to the obvious osmiophilic globule on the thylakoids,×6000. Fig. 11 ATPase activity reaction on the endoplasmic reticulum and karyotin of vein cell for WC treatment, ×15000. Fig. 12 ATPase activity reaction on the tonoplast and chloroplast, while little of osmiophilic globule on the thylakoids of palisade cell for WC treatment, ×8000.

After 12 days of iso-osmotic treatments and calcium regulation, cytochemical localization of ATPase in C. paliurus showed that the ATPase activity was primarily found on the karyon, and in the control treatments it was found on the endoplasmic reticulum (Figs. 3 and 4). ATPase activities were found on the tonoplast for iso-osmotic salt and water treatments (Figs. 5, 6, 9 and 10), and ATPase activities slightly increased under iso-osmotic salt and water treatments. The difference between iso-osmotic treatments was not significant, while osmiophilic globules showed significant increases on the thylakoids under the iso-osmotic water treatment (Fig. 10). For the calcium regulation treatments under iso-osmotic salt and water stress, the ATPase activity was mainly found on the endoplasmic reticulum, golgi apparatus and nuclear membrane (Figs. 7 and 8). For calcium regulation treatment under iso-osmotic water stress, the ATPase activity was mainly found on the endoplasmic reticulum and karyon, and osmiophilic globules significantly decreased (Figs. 11 and 12). In contrast with iso-osmotic treatments without calcium regulation, ATPase activities all increased for iso-osmotic treatments with calcium regulation, and the ATPase activity for iso-osmotic water treatment with calcium regulation was more than that for iso-osmotic salt treatment with calcium regulation (Figs. 7 and 11). These results indicated that the ATPase activity in mesophyll cells of C. paliurus seedlings was related to the mechanism of physiological and ecological response under iso-osmotic salt and water treatments.



Figs. 13-32 Variation for leaf ultrastructure of *C. paliurus* seedlings after 4 days of iso-osmotic NaCl and water stress. Fig. 13 Showing the nucleus of vein cell for CK treatment, ×17000. Fig. 14 Showing the chloroplast of palisade cell for CK treatment, ×17000. Fig. 15 Showing the nuclear membrane, cell wall and plasmalemma of vein cell for S treatment, ×15000. Fig. 16 Showing the thylakoids and intercellular space of palisade cell for S treatment, ×17000. Fig. 17 Showing the nucleus of vein cell for SC treatment, ×15000. Fig. 18 Showing the chloroplast, cell wall, plasmalemma and osmiophilic globule of palisade cell for SC treatment, ×17000. Fig. 19 Showing the nucleus of vein cell for W treatment, ×15000. Fig. 20 Showing the thylakoids, cell wall and plasmalemma of palisade cell for W treatment, ×12000. Fig. 21 Showing the nuclear membrane of vein cell for WC treatment, ×15000. Fig. 22 Showing the chloroplast, plasmalemma, cell wall and vacuole of palisade cell for WC treatment, ×12000.

Ultrastructural variation of leaf under iso-osmotic treatments and calcium regulation

Figures 13 to 32 show that the longer the osmotic stress time, the more serious the injury suffered by seedlings under iso-osmotic treatments. Generally variations of leaf ultrastructure include the disappearance of chloroplast membranes, the swelling of thylakoids, and even the degradation of chloroplasts. Under the same stress time, the injury suffered by seedlings for iso-osmotic salt treatment was less than those exposed to the iso-osmotic water treatment. However, viewing the effect of calcium regulation for iso-osmotic water treatment, the injury suffered by seedlings with calcium regulation was significantly less than those without calcium regulation. For iso-osmotic salt treatment, the degree of injury was not significantly different between with calcium regulation and without calcium regulation. This indicated that given the same degree of iso-osmotic stress, the injury suffered by leaf subcells for iso-osmotic salt treatment was less, while the effect of calcium regulation under iso-water stress was more significant.

Discussion

The enzyme ATPase is a proton or cation pump with major role in the coupling of ATP hydrolysis to proton transport (Porillo 2000; Kuhlbrandt 2004). Regulation of ATPase activity could represent an important cellular mechanism for adversity tolerance (Kalampanayil and Wimmers 2001; Kerkeb et al. 2001; Zhao et al. 2004). An enhancement of the ATPase activity was observed in *C. paliurus* mesophyll cells growing on medium containing NaCl. Reinhold et al. (1984) suggested that the ATPase may act as both a detector and an effector in response to osmotic stress. The ATPase is encoded by a multigene family, and at least 10 isoforms exist in plants (Baxter et al. 2003). Krysan et al. (1996) analyzed T-DNA knockout *Arabidopsis* mutants of ATPase isoforms and demonstrated that at least one ATPase isoform is involved in adversity tolerance. Modulation of this enzyme might occur enzymatically and with transcriptional and translational regulation involvement (Morsomme and Boutry 2000). Osmotic stress can induce mRNA accumulation and/or increase the ATPase enzyme content in some plants (Kalampanayil and Wimmers 2001; Sibole et al. 2005). ATPase was used in this study to determine the changes of this particular enzyme activity in *C. paliurus* exposed to iso-osmotic treatments and calcium regulation. Our results showed an increase of the ATPase activity, which was induced in iso-osmotic medium-treated and calcium-regulated *C. paliurus*. Moreover, the induced ATPase activity for iso-osmotic water treatment with calcium regulation was the highest in all the treatments, followed by the increase of ATPase activity for iso-osmotic treatments with calcium regulation. This implies that calcium regulation plays a stronger role in enhancing the ATPase activity in response to iso-osmotic water stress than in response to iso-osmotic salt stress for *C. paliurus*.

The response of ATPase to iso-osmotic stress was involved in the change of the ATPase activity as well as the variation of the ATPase location (Ma et al. 2003; Peng et al. 2005; Mi et al. 2006). In this study, we determined that the ATPase is generally located on the nucleus under natural growing conditions. Under iso-osmotic salt and water treatments, the ATPase is primarily found on the tonoplasts, and ATPase is transferred onto the nuclear membrane and karyotin under iso-osmotic treatments and calcium regulation. From our results, for the 85.0 mM NaCl or PEG iso-osmotic to 85 mM NaCl treatment, the ATPase was mostly located on the tonoplast, and the injury suffered by leaf ultrastructure was more serious. For the 85.0 mM NaCl or PEG iso-osmotic to 85 mM NaCl + 12mM $Ca(NO_3)_2$ treatment, the ATPase was mainly located on the nucleus and the injury suffered by the leaf ultrastructure was lesser. This suggests that the injury suffered by seedlings was smaller when the ATPase was located on the nucleus, while the injury was greater when the ATPase was located on the tonoplast. Maybe it was significant for early distinguishing of salt and drought-tolerance species to provide a certain theory base.

Osmiophilic globules are considered an index in the function of some lipids, often causing loss of plasmalemma function (Yao 2008). A previous report has indicated that changed osmiophilic globules were connected with the injury degree suffered by plants under adversity stress (Sam et al. 2003). In this study, we found that osmiophilic globules were significantly more numerous and bigger for iso-osmotic treatments than for control treatments, especially, for the iso-osmotic water treatment. By comparingiso-osmotic water treatment with and without calcium regulation, we observed that osmiophilic globules obviously decrease under iso-osmotic water treatment with calcium regulation. The injury suffered by the leaf ultrastructure of *C. paliurus* was lower, suggesting that the injury suffered by *C. paliurus* seedlings was less for iso-osmotic salt treatment than iso-osmotic water treatment, while the effect of calcium regulation under iso-osmotic water stress was better than that of iso-osmotic salt stress. These results ensure that osmiophilic globules could be used as an identifying index for judging the injury degree suffered by plants under iso-osmotic salt and water stress.

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References

Ashraf M, Mcneilly T. 1987. Salinity effects on five cultivars/lines of pearl millet (*Pennisetum americanum* ILl Leeke). *Plant and Soil*, **103**: 13–19.

Baxter I, Tchieu J, Sussman MR, Boutry M, Palmgren MG, Gribskov M, Haeper JF, Axelsen KB. 2003. Genomic comparison of Ptype ATPase ion pumps in *Arabidopsis* and rice. *Plant Physiol*, **132**: 618–628.

Chen Jinhui, Shi Jishen, Gan Xihua. 2006. Ultra-cytochemical localization of ATPase activity in somatic embryogenesis of *Liriodendron hybrid* (*Liriodendron chinese*×*L.tulipifera*). *Acta Botania Boreali-Occidentalia Sinica*, **26**(1): 12–17 (in Chinese).

Epimashko S, Fischer-Schliebs E, Christian AL, Thiel G, Lüttge U. 2006. Na^+/H^+-transporter, H^+-pumps and an aquaporin in light and heavy tonoplast membranes from organic acid and NaCl accumulating vacuoles of the annual facultative CAM plant and halophyte *Mesembryanthemum crystallinum* L. *Planta*, **224**: 944–951.

Fang Shengzuo, Fu Xiangxiang. 2007. Research progress and prospects on silviculture and utilization of *Cyclocarya paliurus* resources. *Journal of Nanjing Forestry University*, **31**(1): 95–100. (in Chinese).

Fang S, Wang J, Wei Z, Zhu Z. 2006. Methods to break seed dormancy in *Cyclocarya paliurus* (Batal)Iljinskaja. *Scientia Horticulturae*, **110**: 305–309.

Greenway H, Munns R. 1980. Mechanisms of salt tolerance in nonhalophytes. *Plant Physiol*, **31**: 149–190.

Hasegawa PM, Bressnan RA, Zhu JK. 2000. Plant cellular and molecular responses to high salinity. *Plant Physiol*, **51**: 463–499.

Hoagland DR, Arnon DI. 1950. The water culture method for growing plants without soil. Circ., California Agricultural Experimental Station, Berkeley, Calif, pp347.

Joshi PA, Stewart JM, Graham ET. 1988. Ultrastructural localization of ATPase activity in cotton fiber during elongation. *Protoplasma*, **143**: 1–10.

Kalampanayil BD, Wimmers LE. 2001. Identification and characterization of a salt-stress-induced plasma membrane H^+-ATPase in tomato. *Plant Cell Environ*, **24**: 999–1005.

Kerkeb L, Donaire JP, Rodriguez-Rosales MP. 2001. Plasma membrane H^+-ATPase activity is involved in adaptation of *tomato calli* to NaCl. *Plant Physiol*, **111**: 483–490.

Krysan PJ, Young JC, Tax F, Sussman MR. 1996. Identification of transferred DNA insertions within *Arabidopsis* genome is involved in signal transduction and ion transport. *Proc Natl Acad Sci USA*, **93**: 8145–8150.

Kuhlbrandt W. 2004. Biology, structure and mechanism of P-type ATPases. *Nat Rev Mol Cell Biol*, **5**: 282–295.

Kurihara H, Fukami H, Kusumoto A, Toyoda Y, Shibata H, Matsui Y, Asami S, Tanaka T. 2003. Hypoglycemic action of *Cyclocarya paliurus* (*Batal.*) *Iljinskaja* in normal and diabetic mice. *Biosci Biotechnol Biochem*, **67**(4): 877–880.

Lambers H. 2003. Dryland salinity: a key environmental issue in southern Australia. *Plant and Soil*, **257**: 5–7.

Li Lei, Xie Mingyong, Yi Xing. 2002. Study on reducing blood sugar of polysaccharide from *Cyclocarya paliurus*. *Chin Med*, **25**(1): 39–41. (in Chinese).

Martinez-Ballesta MC, Martinez V, Carvajal M. 2003. Aquaporin functionality in relation to H^+-ATPase activity in root cells of *Capsicum annuum* grown under salinity. *Plant Physiol*, **117**: 413–420.

Mi Haili, Zhen Guoqi, Xu Xing. 2006. Effects of NaCl stress on plasmalemma and tonoplast H^+-ATPase activities in seedlings roots of *Lycium barbarum* in Ningxia Hui Autonomous region. *Acta Botania Boreali-Occidentalia Sinica*, **26**(4): 748–752. (in Chinese).

Morsomme P, Boutry M. 2000. The plant plasma membrane H^+-ATPase: structure, function and regulation. *Biochim Biophys Acta*, **1465**: 1–16.

Peng FR, Guo J, Wang GP. 2004. Subcellular localization of vegetative storage protein of *Ginkgo biloba*. *Acta Botanica Sinica*, **46**(1): 77–85.

Porillo F. 2000. Regulation of plasma membrane H^+-ATPase in fungi and plants. *Biochim Biophy Acta*, **1469**: 31–42.

Qiu QS, Guo Y, Quintero FJ. 2004. Regulation of vacuolar Na^+/H^+ pumping and plasma membrane H^+- ATPase activity in proteoid roots of white lupin under phosphate deficiency. *Plant Physiol*, **279**: 207–215.

Reinhold L, Seiden A, Volokita M. 1984. Is modulation of the rate of proton pumping a key event in osmoregulation. *Plant Physiol*, **75**: 846–849.

Salem E, Micaela C, Rachid G. 2005. Study of the involvement of osmotic adjustment and H^+-ATPase activity in the resistance of *Catharanthus roseus* suspension cells to salt stress. *Plant Cell Tis Org Cul*, **80**: 287–294.

Sam O, Ramírez C, Coronado MJ. 2003. Changes in tomato leaves induced by NaCl stress: leaf organization and cell ultrastructure. *Plant Biol,* **47**: 361–366.

Shi H, Lee BH, Wu SJ, Zhu JK. 2003. Overexpression of a plasma membrane H^+-ATPase antiporter gene improves salt tolerance in *Arabidopsis thaliana*. *Nat Biotechnol*, **21**: 81–85.

Sibole JV, Cabot C, Michalke W, Poschenrieder C, Barcelo J. 2005. Relationship between expression of the PM H^+-ATPase, growth and ion partitioning in the leaves of salt-treated *Medicago* species. *Planta*, **221**: 557–566.

Wang BS, Lüttge U, Rataj R. 2001. Effects of salt treatment and osmotic stress on V-ATPase and V-PPase in leaves of the halophyte *Suaeda salsa*. *J Exp Bot*, **52**: 2355–2365.

Wang YZ, Sze H. 1985. Similarities and differences between the tonoplast-type and mitochondrial H^+-ATPase of oat roots. *J Biol Chem*, **260**: 10434–10443.

Xie Mingyong, Li Lei. 2001. Review in studies on chemical constituents and bioactivities of *Cyclocarya paliurus*. Chin. Tradition. *Herb Drugs*, **32**(4): 365–366 (in Chinese).

Yang YL, Zhang F, Zhao MG, An LZ, Zhang LX, Chen NL. 2007. Properties of plasma membrane H^+-ATPase in salt-treated *Populus euphratica callus*. *Plant Cell Rep*, **26**: 229–235.

Yao Ruiling, Fang Shengzuo. 2007. Effect of NaCl stress and Calcium regulation on ion distribution in root tissue of *Cyclocarya paliurus*. *Journal of Plant Resource and Environment*, **16**(2): 22–26 (in Chinese).

Yao Ruiling, Wang Yin, Fang Shengzuo. 2008. Cytochemical localization of the H^+-ATPase and variation of ultra-structure in leaves of *Cyclocarya paliurus* seedlings under salt-stress. *Plant physiology communication*, **44**(2): 206–210. (in Chinese).

Zhao KF, Fan H, Zhou S, Song J. 2003. Study on the salt and drought tolerance of *Suaeda salsa* and *Kalanchoe claigremontiana* under iso-osmotic salt and water stress. *Plant Sci*, **165**: 837–844.

Zhao LQ, Zhang F, Guo JK, Yang YL, Li BB, Zhang LX. 2004. Nitric oxide functions as a signal in salt resistance in the calluses from two ecotypes of reed. *Plant Physiol*, **134**: 849–857.